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# Association of DNA Mismatch Repair and Mutations in *BRAF* and *KRAS* With Survival After Recurrence in Stage III Colon Cancers

## A Secondary Analysis of 2 Randomized Clinical Trials

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**IMPORTANCE** The association of biomarkers with patient survival after recurrence (SAR) of cancer is poorly understood but may guide management and treatment.

**OBJECTIVE** To determine the association of DNA mismatch repair (MMR) status and somatic mutation in the B-Raf proto-oncogene (c.1799T>A [V600E]; *BRAF*$^{V600E}$) or exon 2 of the KRAS proto-oncogene (*KRAS*) in the primary tumor with SAR in patients with stage III colon carcinomas treated with adjuvant chemotherapy.

**DESIGN, SETTING, AND PARTICIPANTS** Patients with resected stage III colon cancers were randomized to adjuvant FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin) chemotherapy with or without cetuximab (North Central Cancer Treatment Group N0147 trial) or adjuvant FOLFOX chemotherapy with or without bevacizumab (National Surgical Adjuvant Breast and Bowel Project C-08 trial). Associations of biomarkers with SAR were analyzed using Cox proportional hazards models adjusted for clinicopathologic features and time to recurrence (data collected February 10, 2004, to August 7, 2015).

**MAIN OUTCOMES AND MEASURES** The primary study outcome was survival after recurrence of cancer. A secondary outcome measure was the effect of the site of the primary tumor on the association of biomarkers with SAR.

**RESULTS** Among 871 patients with cancer recurrence in the N0147 trial (472 men [54.2%] and 399 women [45.8%]; mean [SD] age, 57.8 [11.2] years) and 524 in the C-08 trial (269 men [51.3%] and 255 women [48.7%]; mean [SD] age, 57.0 [11.7] years), multivariable analysis revealed that patients whose tumors had deficient vs proficient MMR had significantly better SAR (adjusted hazard ratio [AHR], 0.70; 95% CI, 0.52-0.96; $P = .03$). Patients whose tumors harbored mutant *BRAF*$^{V600E}$ (AHR, 2.45; 95% CI, 1.85-3.25; $P < .001$) or mutant *KRAS* (AHR, 1.21; 95% CI, 1.00-1.47; $P = .052$) had worse SAR compared with those whose tumors had wild-type copies of both genes, although only results for *BRAF*$^{V600E}$ achieved statistical significance. Significant interactions were found for MMR ($P = .03$) and *KRAS* ($P = .02$) by primary tumor site for SAR. Improved SAR was observed for patients with deficient MMR tumors of the proximal vs distal colon (AHR, 0.57; 95% CI, 0.40-0.83; $P = .003$), and worse SAR was observed for tumors of the distal colon with mutant *KRAS* in codon 12 (AHR, 1.76; 95% CI, 1.30-2.38; $P < .001$) and codon 13 (AHR, 1.76; 95% CI, 1.08-2.86; $P = .02$).

**CONCLUSIONS AND RELEVANCE** In patients with recurrence of stage III colon cancer, deficient MMR was significantly associated with better SAR, and this benefit was limited to primary tumors of the proximal colon. Mutations in *BRAF*$^{V600E}$ were significantly associated with worse SAR, and worse SAR for *BRAF*$^{V600E}$ or *KRAS* mutant tumors was more strongly associated with distal cancers. These biomarkers have implications for patient management at recurrence.

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Prognostic biomarkers in patients with tumor recurrence have the potential to influence management and treatment decisions. Approximately 30% of patients with stage III colon carcinoma will experience recurrence of their disease despite adjuvant chemotherapy.[1] Studies have shown that DNA mismatch repair (MMR) status and mutations in the B-Raf proto-oncogene with a c.1799T>A (V600E) mutation (*BRAF*$^{V600E}$ [HGNC 1097]) or the KRAS proto-oncogene (*KRAS*) [HGNC 6407]) can provide prognostic information in patients with stage III disease.[2] However, the association of biomarkers with survival after recurrence (SAR) remains poorly understood, and studies have been underpowered given the relatively low frequency of these alterations and modest rates of tumor recurrence.

In patients with stage III tumors who participated in adjuvant chemotherapy trials, those whose tumors showed deficient MMR (dMMR) or microsatellite instability (MSI) have generally had better clinical outcomes compared with those with proficient MMR (pMMR) or microsatellite stability.[3] However, the association of dMMR or MSI with prognosis is less robust in stage III vs stage II disease,[4] and limited data exist in patients treated with the adjuvant FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin) regimen in contrast to fluorouracil alone.[5-8] As with metastatic disease,[9] *BRAF*$^{V600E}$ mutations have been shown to be significantly associated with poorer survival,[10-12] with a stronger impact seen for overall survival compared with disease-free or progression-free survival[13] for reasons that remain unclear. Because *BRAF*$^{V600E}$ mutations are significantly enriched in sporadic colon cancers with dMMR or MSI (owing to epigenetic inactivation of *MLH1*),[14,15] the combined MMR *BRAF* variable may be more informative than either alone. In this regard, a new consensus guideline for the molecular testing of colorectal cancer recommends that *BRAF* be analyzed in conjunction with MMR for prognostic stratification. Data for the association of a *KRAS* mutation with clinical outcome have been less consistent than for a *BRAF*$^{V600E}$ mutation.[13,16-18] In participants in the North Central Cancer Treatment Group (NCCTG) N0147[16] and the Pan European Trial Adjuvant Colon Cancer-8 (PETACC-8) adjuvant chemotherapy trials,[19] stage III colon cancers with mutant vs wild-type (WT) *KRAS* had poorer rates of disease-free survival.

We studied the association of MMR and mutations in *BRAF*$^{V600E}$ or *KRAS* in the primary tumor with SAR in participants in the NCCTG N0147 and the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-08 adjuvant chemotherapy trials. These trials evaluated FOLFOX chemotherapy alone or combined with cetuximab (N0147)[20] or bevacizumab (NSAPB C-08),[21] wherein neither antibody significantly improved patient outcome vs FOLFOX chemotherapy alone. We also determined whether the association of biomarkers with SAR depended on the primary tumor site within the colon given recent data suggesting prognostic differences by tumor site.[6,22]

### Key Points

**Question** What is the association of DNA mismatch repair and mutation in the B-Raf proto-oncogene (c.1799T>A [V600E] [*BRAF*$^{V600E}$]) or the KRAS proto-oncogene (*KRAS*) with survival after recurrence in patients with stage III colon carcinoma treated in 2 trials of folinic acid (leucovorin calcium), fluorouracil, and oxaliplatin (FOLFOX)–based adjuvant chemotherapy?

**Findings** In a secondary analysis of 2 randomized clinical trials that include 1395 patients with cancer recurrence, tumors with deficient vs proficient mismatch repair had a 30% reduction in mortality. Mutations in *BRAF*$^{V600E}$, but not *KRAS*, were associated with shorter survival after recurrence in the overall cohort.

**Meaning** DNA mismatch repair and *BRAF* mutations can provide prognostic information in patients with tumor recurrence.

## Methods

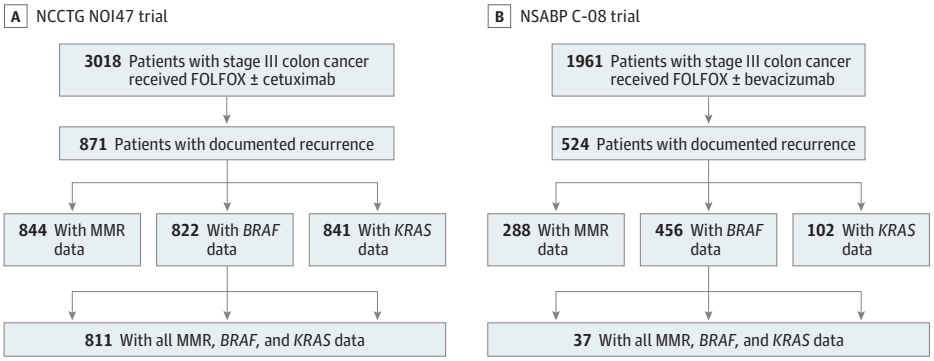
The study population consisted of patients with stage III adenocarcinoma of the colon who developed recurrence during participation in the phase III adjuvant chemotherapy studies NCCTG N0147 (n = 871)[20] and NSABP C-08 (n = 524).[21] The analysis was prespecified in the study protocols. We categorized primary tumor site as proximal to or at or distal to the splenic flexure. Each trial was approved by the respective institutional review boards[20,21] and by the NCCTG (now part of Alliance for Clinical Trials in Oncology) or NSABP (now part of NRG Oncology). Each participant signed an institutional review board-approved, protocol-specific informed consent document. Data quality was ensured by review by the Statistics and Data Center of the Alliance for Clinical Trials in Oncology or NRG Oncology and by the study chairpersons (C.J.A. and S.R.A.) per established policies.

### Molecular Testing

We collected data from February 10, 2004, to August 7, 2015. The DNA MMR proteins MutL protein homolog 1 (MLH1), MutS protein homolog 2 (MSH2), and MutS protein homolog 6 (MSH6) were analyzed in formalin-fixed, paraffin-embedded tumor tissues from the N0147 trial as previously described[10]; MLH1 and MSH2 expression were analyzed in tumors from the C-08 trial as reported.[23] Loss of MMR protein was defined as the absence of nuclear staining in tumor cells in the presence of nuclear staining in normal colonic epithelium and lymphocytes. Tumors with loss of an MMR protein were categorized as having dMMR and those with intact expression as having pMMR. All biomarker assays were interpreted with investigators blinded to patient outcomes.

Mutation status of *BRAF*$^{V600E}$ and *KRAS* were determined using genomic DNA extracted from macrodissected formalin-fixed, paraffin-embedded tumor tissue collected prospectively. In the N0147 trial, testing for the *BRAF*$^{V600E}$ mutation in exon 15 was performed using a multiplex allele-specific, polymerase chain reaction–based assay and an automated sequencing technique, as previously described.[10] Mutation status in *KRAS* exon 2 was analyzed using a *KRAS* mutation kit (DxS; TheraScreen) assessing for 7 different mutations in codons 12 and 13.[16] In the N0147 trial, molecular analyses were performed in a Clinical Laboratory Improvement Amendments–compliant laboratory. Mutation profiling of tumor specimens from the C-08 trial was performed using

Figure 1. Flow Diagrams of the Study Population




Median follow-up among surviving patients was 6.0 (interquartile range [IQR], 5.2-7.0) years in the North Central Cancer Treatment Group (NCCTG) N0147 trial and 6.3 (IQR, 6.0-6.9) years in the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-08 trial. *BRAF* indicates B-Raf proto-oncogene; FOLFOX, folinic acid (leucovorin calcium), fluorouracil, and oxaliplatin; *KRAS*, KRAS proto-oncogene; and MMR, DNA mismatch repair.

2 panel assays (OncoCarta and ColoCarta; Sequenom), with the running of samples on the mass spectrometry platform as described previously.[23]

### Statistical Analysis

Survival after recurrence, defined as the time from recurrence to death from any cause, was the primary study outcome. Owing to the potential for significant confounding, all analyses were based on multivariable models that were adjusted for clinicopathologic variables, time to recurrence (TTR), and biomarkers. The distribution of SAR between patient subgroups by biomarkers was estimated based on direct adjusted survival curves.[24-26] Because initial results showed significant differences in SAR among the 4 arms of the 2 adjuvant chemotherapy trials ($P = .03$), multivariable Cox proportional hazards models (stratified by the 4 treatment groups) were applied to assess the effect of biomarkers on SAR among patients with recurrence. Models were adjusted for age, sex, performance score, initial T/N stage, histologic grade, time from initial treatment to recurrence, primary tumor site, and biomarkers when applicable. The proportional hazards assumption was confirmed by examination of the Schoenfeld residuals plot.[27] We determined the interaction effects of the primary tumor site with the effect of biomarkers on SAR. Subgroup analyses were performed when statistically significant interaction effects were present. Association analyses were performed in patients from the modified sixth version of FOLFOX (mFOLFOX6)–alone treatment arms from both studies owing to clinical relevance. Two-sided *P* values are reported; $P < .05$ was considered statistically significant and was not adjusted for multiple comparisons. Analyses were performed using SAS software (version 9.4; SAS Institute Inc).

## Results

Among the adjuvant trial participants, 3018 patients received mFOLFOX6 with or without cetuximab (N0147 trial)[20] and 1961 patients received mFOLFOX6 with or without bevacizumab (C-08 trial).[21] At a median follow-up of 6.0 (interquartile range [IQR], 5.2-7.0) years (N0147 trial) and 6.3 (IQR, 6.0-6.9) years (C-08 trial), 871 patients from the N0147 trial (472 men [54.2%] and 399 women [45.8%]; mean [SD] age, 57.8 [11.2] years) and 524 patients from the C-08 trial (269 men [51.3%] and 255 women [48.7%]; mean [SD] age, 57.0 [11.7] years) had a documented first recurrence and are included in this secondary analysis. Among these patients, 848 had complete and available data on MMR and the mutational status of the *BRAF* and *KRAS* genes (**Figure 1**).

### Molecular Markers and SAR

The multivariable associations of patient demographics and clinicopathologic features, adjusted for biomarkers (MMR, *KRAS*, and *BRAF*), with SAR are presented in **Table 1**. Patients with distal tumors had significantly better SAR than did patients with proximal tumors (adjusted hazard ratio [AHR], 0.70; 95% CI, 0.58-0.84; $P < .001$). Longer TTR after primary resection was associated with significantly better SAR (AHR for 1-year delay, 0.79; 95% CI, 0.72-0.87; $P < .001$) (Table 1). In addition, a significant association with SAR was found for patient performance score (AHR, 1.23; 95% CI, 1.01-1.49; $P = .04$), N stage (AHR, 1.39; 95% CI, 1.17-1.66; $P < .001$), and histologic grade (AHR, 1.40; 95% CI, 1.17-1.68; $P < .001$). Among patients who experienced recurrence, those whose tumors showed pMMR vs dMMR (AHR, 1.71; 95% CI, 1.32-2.21; $P < .001$) or had WT *KRAS* (AHR, 1.22; 95% CI, 1.05-1.14; $P = .01$) and WT *BRAF* (AHR, 1.47; 95% CI, 1.17-1.83; $P < .001$) vs either mutated gene had significantly longer median TTR (eTable 1 in the Supplement).

Multivariable associations of molecular markers with SAR are shown in **Table 2**. After adjustment for covariates, including TTR after primary treatment, patients with dMMR vs pMMR tumors had significantly better SAR (AHR, 0.70; 95% CI, 0.52-0.96; $P = .03$) (**Figure 2**A and Table 2). Patients whose tumors had mutant $BRAF^{V600E}$ had significantly worse SAR compared with those whose tumors had WT *BRAF* (AHR, 2.45; 95% CI, 1.85-3.25; $P < .001$) (Figure 2B and C and Table 2). Given that MMR status and $BRAF^{V600E}$ are strongly associated, we analyzed MMR and *BRAF* as a combined variable. Patients had similarly poor adjusted median SAR times of 14.5 (95% CI, 11.8-45.9) months (AHR, 1.52; 95% CI, 0.99-2.34; $P = .06$) if their tumor had dMMR plus mutant $BRAF^{V600E}$ and 15.4 (95% CI, 10.8-16.7) months if their tumor had pMMR plus mutant $BRAF^{V600E}$ (AHR, 2.64; 95% CI, 1.96-3.57; $P < .001$), which was

Table 1. Multivariable Associations of Patient Demographics and Disease Characteristics With Survival After Recurrence [a]

| Characteristic | Patients, No. (%)[b] | AHR (95% CI) | *P* Value |
|---|---|---|---|
| Age per 10-y increase | 832 (100) | 1.06 (0.98-1.15) | .17 |
| Sex | | | |
| Male | 445 (53.5) | 1 [Reference] | NA |
| Female | 387 (46.5) | 0.90 (0.76-1.06) | .21 |
| Performance score[c] | | | |
| 0 | 629 (75.6) | 1 [Reference] | NA |
| 1 | 198 (23.8) | 1.23 (1.01-1.49) | .04 |
| 2 | 5 (0.6) | 7.97 (3.19-19.88) | <.001 |
| T stage | | | |
| T1/2 | 47 (5.6) | 1 [Reference] | NA |
| T3 | 631 (75.8) | 1.32 (0.88-1.99) | .18 |
| T4 | 154 (18.5) | 1.41 (0.91-2.19) | .13 |
| N stage | | | |
| N1 | 339 (40.7) | 1 [Reference] | NA |
| N2 | 493 (59.3) | 1.39 (1.17-1.66) | <.001 |
| Primary tumor site | | | |
| Distal | 384 (46.2) | 0.70 (0.58-0.84) | <.001 |
| Proximal | 448 (53.8) | 1 [Reference] | NA |
| Histologic grade | | | |
| Low (1-2)[d] | 587 (70.6) | 1 [Reference] | NA |
| High (3-4)[e] | 245 (29.4) | 1.40 (1.17-1.68) | <.001 |
| Time to recurrence per 1-y increase | 832 (100) | 0.79 (0.72-0.87) | <.001 |

Abbreviations: AHJR, adjusted hazard ratio; *BRAF*, B-Raf proto-oncogene; *KRAS*, KRAS proto-oncogene; MMR, mismatch repair; NA, not applicable.

[a] Multivariable model in 832 patients includes complete data on all covariates (age, sex, performance score, T stage, N stage, primary tumor site, histologic grade, time to recurrence, MMR, *KRAS*, and *BRAF*. The AHRs, 95% CIs, and *P* values associated with MMR, *KRAS*, and *BRAF* are presented in Table 2. Stratified Cox proportional hazards models are used with 4 treatment arms as individual strata.

[b] Percentages have been rounded and may not total 100.

[c] Higher scores indicate worsening patient functional status.

[d] Indicates good and moderate differentiation, respectively.

[e] Indicates poor differentiation and undifferentiated, respectively.

shorter SAR compared with pMMR and WT *BRAF* (median, 28.4 months; 95% CI, 26.2-31.9 months) (Table 2 and Figure 2D). In contrast, patients showed better adjusted median SARs of 30.3 (95% CI, 21.4 to not reached) if their tumor had dMMR with WT *BRAF* and 28.4 (95% CI, 26.2-31.9) months if their tumor had pMMR with WT *BRAF*, with no statistical difference (*P* = .43) between these 2 groups (Table 2 and Figure 2D). Within the subset of dMMR tumors, we observed that those with *BRAF* mutations had significantly poorer SAR compared with those with WT *BRAF* (AHR, 2.70; 95% CI, 1.23-5.93; *P* = .01) (Table 2). Patients whose tumors harbored *KRAS* exon 2 mutations had shorter SAR (25.9 [95% CI, 23.5-29.4] months) compared with those whose tumors were WT *KRAS* and *BRAF* (32.1 [95% CI, 27.6-37.1] months) (*P* = .052) (Table 2). When *KRAS* was analyzed by codon 12 (median SAR, 23.8 [95% CI, 21.2-27.2] months) or codon 13 mutations (median SAR, 27.2 [95% CI, 22.0-33.8] months) vs WT *KRAS*, the associations did not reach statistical significance (*P* = .08 for *KRAS* analyzed by codon 12 mutations and *P* = .17 for *KRAS* analyzed by codon 13 mutations) (Table 2). Patients whose tumors had WT *BRAF* and WT *KRAS* had the longest SAR (adjusted median, 32.1 [95% CI, 27.6-37.1] months) of all groups that was significantly improved compared with patients whose tumors had $BRAF^{V600E}$ mutation (adjusted median SAR, 15.0 [95% CI, 12.1-16.9] months) (*P* < .001) (Table 2).

## Analysis by Primary Tumor Site

Based on statistically significant interactions between biomarkers and primary tumor site for SAR (Table 2), we separately examined the associations between biomarkers and SAR among patients with proximal or distal tumors (**Table 3** and eFigure in the Supplement). After adjustment for covariates, patients with dMMR tumors of the proximal but not the distal colon had significantly better SAR (AHR, 0.57; 95% CI, 0.40-0.83; *P* = .003) (Table 3 and eFigure, A in the Supplement), with *P* = .03 for interaction (Table 2). Patients with $BRAF^{V600E}$-mutated tumors had significantly shorter SAR for proximal tumors (AHR, 1.90; 95% CI, 1.37-2.64; *P* < .001) (eFigure, D in the Supplement) and distal tumors (AHR, 5.84; 95% CI, 3.27-10.43; *P* < .001) vs those whose tumors had WT *BRAF* (eFigure, B in the Supplement) or WT *BRAF* and WT *KRAS* (eFigure, C in the Supplement) (Table 3), although the interaction between *BRAF* and primary tumor site for SAR did not achieve statistical significance (*P* = .056) (Table 2). A significant interaction test was observed for *KRAS* mutations (codons 12 and 13) (*P* = .02) and the combined *KRAS-BRAF* variable (*P* < .001) with primary tumor site for SAR (Table 2). Compared with tumors with WT *KRAS*, patients whose tumors harbored *KRAS* mutations at codon 12 (AHR, 1.76; 95% CI, 1.30-2.38; *P* < .001) or codon 13 (AHR, 1.76; 95% CI, 1.08-2.86; *P* = .02) each had significantly worse SAR among those with distal cancer but not for those with proximal cancers with *KRAS* mutations at codon 12 (AHR, 0.85; 95% CI, 0.65-1.12; *P* = .26) or codon 13 (ARH, 0.91; 95% CI, 0.61-1.36; *P* = .65) (Table 3 and eFigure, C in the Supplement). For the combined MMR and *BRAF* variable, the adjusted median SAR was shorter for patients with dMMR and mutant $BRAF^{V600E}$ tumors of the distal vs proximal colon (5.7 [95% CI, 2.8 to not reached] vs 14.5 [95% CI, 6.6 to not reached] months). Furthermore, dMMR and mutant $BRAF^{V600E}$ tumors in the distal colon had significantly

Table 2. Adjusted Associations Between Biomarkers and SAR

| Biomarker | No. of Events/Patients | Adjusted Median SAR (95% CI), mo[a] | AHR (95% CI)[b] | *P* Value | *P* Value for Interaction With Tumor Site | *P* Value for Interaction With Treatment |
|---|---|---|---|---|---|---|
| MMR | | | | | | |
| dMMR | 54/73 | 32.1 (24.4-NR) | 0.70 (0.52-0.96) | .03 | .03 | .003 |
| pMMR | 521/759 | 25.1 (23.3-28.3) | 1 [Reference] | NA | | |
| *KRAS* | | | | | | |
| Codon 12 MT | 201/275 | 23.8 (21.2-27.2) | 1.20 (0.98-1.47) | .08 | .02 | .23 |
| Codon 13 MT | 55/68 | 27.2 (22.0-33.8) | 1.24 (0.91-1.67) | .17 | | |
| WT | 319/489 | 28.1 (24.1-32.3) | 1 [Reference] | | | |
| *BRAF* | | | | | | |
| MT | 102/112 | 14.5 (10.8-16.7) | 2.45 (1.85-3.25) | <.001 | .06 | .57 |
| WT | 473/720 | 28.7 (26.8-32.1) | 1 [Reference] | | | |
| *KRAS* and/or *BRAF* | | | | | | |
| *BRAF* MT | 102/112 | 15.0 (12.1-16.9) | 2.45 (1.85-3.25) | <.001 | <.001 | .67 |
| *KRAS* MT | 256/343 | 25.9 (23.5-29.4) | 1.21 (1.00-1.47) | .052 | | |
| Both WT | 217/377 | 32.1 (27.6-37.1) | 1 [Reference] | | | |
| MMR + *BRAF* | | | | | | |
| MT *BRAF* dMMR | 28/33 | 14.5 (11.8-45.9) | 1.52 (0.99-2.34) | .06 | .08 | .02 |
| WT *BRAF* dMMR | 26/40 | 30.3 (21.4-NR) | 0.85 (0.56-1.28) | .43 | | |
| MT *BRAF* pMMR | 74/79 | 15.4 (10.8-16.7) | 2.64 (1.96-3.57) | <.001 | | |
| WT *BRAF* pMMR | 447/680 | 28.4 (26.2-31.9) | 1 [Reference] | NA | | |
| *BRAF* in patients with dMMR[c] | | | | | | |
| MT *BRAF* | 28/33 | NC | 2.70 (1.23-5.93) | .01 | NA | NA |
| WT *BRAF* | 26/40 | NC | 1 [Reference] | NA | | |

Abbreviations: AHR, adjusted hazard ratio; *BRAF*, B-Raf proto-oncogene; dMMR, deficient DNA mismatch repair; *KRAS*, KRAS proto-oncogene; MT, mutant; NA, not applicable; NC, not calculated; NR, not reached; pMMR, proficient MMR; SAR, survival after recurrence; WT, wild-type.

[a] Based on direct adjusted survival curves from the Cox proportional hazards model.

[b] Adjusted for age, sex, performance score, T/N stage, primary tumor site, histologic grade, biomarkers (when applicable), and time to recurrence. Stratified Cox proportional hazards models are used with 4 treatment arms as individual strata.

[d] Median not calculated owing to small sample size (model convergence failed).

shorter SAR than did patients with pMMR and WT *BRAF* tumors (AHR, 9.38; 95% CI, 3.23-27.28; $P < .001$) (Table 3 and eFigure, D in the Supplement).

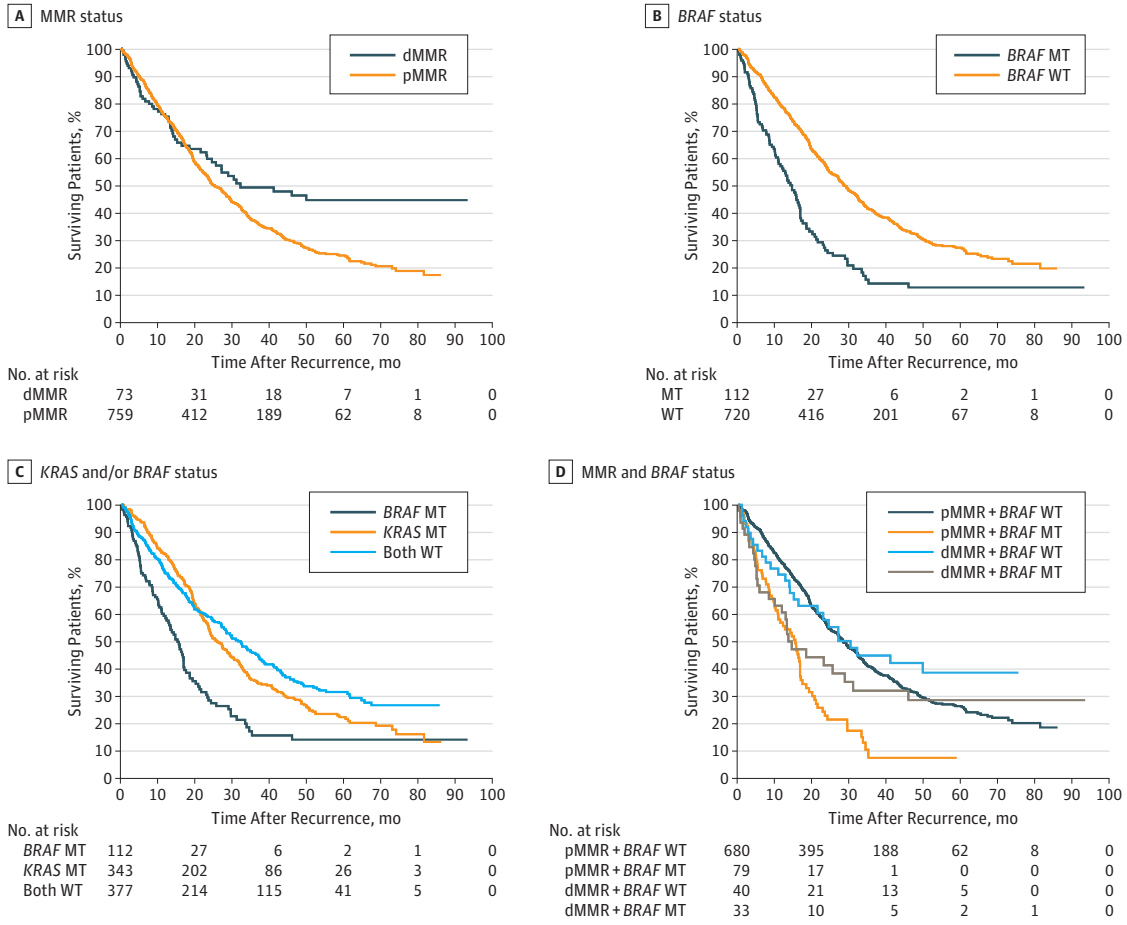
### Analysis by Study Treatment Arm

A statistically significant interaction was observed between the study treatment arm and MMR status ($P = .003$) and for the combined variable of MMR and *BRAF* ($P = .02$) for SAR (Table 2). The significantly favorable effect of dMMR on SAR shown in multivariable analysis was evident in the study arms receiving FOLFOX alone from both adjuvant trials (HR, 0.50; 95% CI, 0.31-0.81; $P = .004$) but was not observed in the FOLFOX + cetuximab arm of the N0147 trial (HR, 1.19; 95% CI, 0.78-1.82; $P = .43$) (eTable 2 in the Supplement). Overall, an association of mutant $BRAF^{V600E}$ with significantly poorer SAR was observed in patients treated with FOLFOX alone (HR, 2.50; 95% CI, 1.66-3.78; $P < .001$) or with cetuximab (HR, 2.50; 95% CI, 1.66-3.75; $P < .001$). However, patients whose cancers were dMMR and had mutant $BRAF^{V600E}$ showed significantly poorer SAR when cetuximab was added to FOLFOX (HR, 2.95; 95% CI, 1.64-5.32; $P < .001$) but not in patients whose tumors were treated with FOLFOX alone (HR, 1.03; 95% CI, 0.52-2.0; $P = .94$) (eTable 2 in the Supplement). A similar effect was observed for tumors with *KRAS* codon 12 mutations whereby their SAR was worse than in patients with WT *KRAS* tumors when treated with FOLFOX plus cetuximab (HR, 1.39; 95% CI, 1.09-1.93; $P = .05$) but not when treated with FOLFOX alone (HR, 1.22; 95% CI, 0.93-1.62; $P = .15$) (eTable 2 in the Supplement). Among patients with *KRAS* WT tumors, no differences in SAR were observed within proximal or distal primary tumors by treatment arm.

## Discussion

We determined the effect of biomarkers on SAR in patients with stage III colon cancer who participated in 2 large adjuvant chemotherapy trials of FOLFOX-containing therapy. In the overall cohort, patients whose tumors had mutant *BRAF* had significantly worse SAR, with a 14.2-month decrease in adjusted median survival time compared with patients whose tumors had WT *BRAF*. This result can explain, at least in part, prior data showing that mutant $BRAF^{V600E}$ was more strongly associated with overall survival compared with disease-free survival or relapse-free survival in the N0147[6] and PETACC-3 adjuvant chemotherapy trials.[13] Furthermore, these findings suggest that the effect of $BRAF^{V600E}$ mutation on tumor aggressiveness is enhanced at the time of tumor recurrence

Figure 2. Associations of Tumor Status and Survival After Recurrence




In patients with stage III colon carcinoma treated with FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin)–containing adjuvant therapy, direct adjusted plots of survival after recurrence are shown by deficient DNA mismatch repair (dMMR) and proficient MMR (pMMR) status (A), mutated (MT) vs wild-type (WT) B-Raf proto-oncogene (*BRAF*) (B), and the combined variables of KRAS proto-oncogene (*KRAS*) and *BRAF* (C) and MMR and *BRAF* (D).

because recurrence of these tumors led to accelerated patient mortality. In this regard, patients whose tumors harbored *BRAF*$^{V600E}$ mutations had an approximately 3-fold increase in early peritoneal metastases compared with those patients whose tumors showed WT *BRAF* in the N0147 cohort with stage III tumors.[28] These data are consistent with other reports showing adverse outcomes[29] and significantly higher rates of peritoneal and distant lymph node metastases among *BRAF*$^{V600E}$ mutant metastatic colorectal cancers.[9]

Among patients with dMMR tumors, we found that their adjusted median SAR was 7 months longer than that for patients with pMMR tumors, indicating a clinically significant survival advantage for this patient subset. This finding is consistent with the longer recurrence-free interval (ie, TTR) observed for dMMR vs pMMR tumors in the overall study cohort. The analysis was adjusted for covariates that included *BRAF* mutation status, TTR, and primary tumor site, which were the variables whose inclusion in the multivariable model had the greatest effect on SAR in dMMR tumors. The longer SAR for patients with dMMR tumors may be explained, in part, by the increase in recurrence rates at regional vs distant sites, such as the liver, that was observed in the N0147 cohort.[28] Among patients whose tumors had mutant *KRAS*, a poorer SAR that did not reach statistical significance was observed for codon 12 or 13 mutations.

Sporadic colon cancers with dMMR are highly enriched with *BRAF*$^{V600E}$ mutations,[5,14] and a forthcoming consensus guideline[30] recommends that *BRAF*$^{V600E}$ mutation testing be performed in conjunction with MMR analysis for prognostic stratification. A similarly poor SAR was observed for patients with *BRAF*$^{V600E}$-mutant dMMR cancer (adjusted median SAR, 14.5 months; 95% CI, 11.8-45.9 months) and pMMR cancers (adjusted median SAR, 15.4 months; 95% CI, 10.8-16.7 months). In contrast, patients whose tumors had WT *BRAF* showed significantly better adjusted median SARs of 30.3 months (95% CI, 21.4 months to not reached) for dMMR and 28.4 months (95% CI, 26.2-31.9 months) for pMMR cancers. Therefore, the mutational status of *BRAF* is an important determinant of SAR that confers adverse outcomes in patients with dMMR and pMMR cancers. In a pooled analysis of patients with stage II

Table 3. Adjusted Associations Between Biomarkers and SAR Among Patients by Primary Tumor Site

| Biomarker | Proximal Tumor | | | | Distal Tumor | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of Events/ Patients | Adjusted Median SAR (95% CI), mo[a] | AHR (95% CI)[b] | *P* Value | No. of Events/ Patients | Adjusted Median SAR (95% CI), mo[a] | AHR (95% CI)[b] | *P* Value |
| MMR | | | | | | | | |
| dMMR | 41/55 | 30.3 (16.3-NR) | 0.57 (0.40-0.83) | .003 | 13/18 | 27.0 (24.4-NR) | 1.26 (0.69-2.28) | .45 |
| pMMR | 308/393 | 19.0 (17.2-20.6) | 1 [Reference] | NA | 213/366 | 36.6 (33.3-43.3) | 1 [Reference] | NA |
| *KRAS* | | | | | | | | |
| Codon 12 MT | 120/162 | 21.8 (19.7-25.3) | 0.85 (0.65-1.12) | .26 | 81/113 | 27.0 (23.2-34.4) | 1.76 (1.30-2.38) | <.001 |
| Codon 13 MT | 35/43 | 21.9 (16.6-30.2) | 0.91 (0.61-1.36) | .65 | 20/25 | 33.8 (24.4-48.1) | 1.76 (1.08-2.86) | .02 |
| WT | 194/243 | 16.7 (14.5-19.4) | 1 [Reference] | NA | 125/246 | 43.3 (37.1-55.1) | 1 [Reference] | NA |
| *BRAF* | | | | | | | | |
| MT | 85/94 | 13.2 (9.9-16.7) | 1.90 (1.37-2.64) | <.001 | 17/18 | 11.3 (6.5-23.4) | 5.84 (3.27-10.43) | <.001 |
| WT | 264/354 | 21.8 (19.3-23.8) | 1 [Reference] | NA | 209/366 | 39.2 (33.9-44.2) | 1 [Reference] | NA |
| *KRAS* and/or *BRAF* | | | | | | | | |
| *BRAF* MT | 85/94 | 12.8 (9.9-16.0) | 1.90 (1.37-2.64) | <.001 | 17/18 | 16.7 (6.6-25.5) | 5.84 (3.27-10.43) | <.001 |
| *KRAS* MT | 155/205 | 23.3 (21.1-27.2) | 0.87 (0.67-1.12) | .28 | 101/138 | 29.1 (24.5-36.3) | 1.76 (1.32-2.34) | <.001 |
| Both WT | 109/149 | 19.1 (14.7-26.8) | 1 [Reference] | NA | 108/228 | 45.4 (38.5-61.4) | 1 [Reference] | NA |
| MMR + *BRAF* | | | | | | | | |
| MT *BRAF* dMMR | 24/29 | 14.5 (11.8-NR) | 1.08 (0.66-1.75) | .76 | 4/4 | 5.7 (2.8-NR) | 9.38 (3.23-27.28) | <.001 |
| WT *BRAF* dMMR | 17/26 | 30.3 (16.3-NR) | 0.59 (0.35-0.99) | .047 | 9/14 | 27.0 (24.4-NR) | 1.13 (0.56-2.29) | .73 |
| MT *BRAF* pMMR | 61/65 | 15.0 (9.9-16.7) | 1.92 (1.35-2.73) | <.003 | 13/14 | 14.5 (6.6-NR) | 5.38 (2.81-10.31) | <.001 |
| WT *BRAF* pMMR | 247/328 | 20.7 (19.0-23.5) | 1 [Reference] | NA | 200/352 | 39.2 (34.4-44.2) | 1 [Reference] | NA |

Abbreviations: AHR, adjusted hazard ratio; *BRAF*, B-Raf proto-oncogene; dMMR, deficient DNA mismatch repair; *KRAS*, KRAS proto-oncogene; MT, mutant; NA, not applicable; NR, not reached; pMMR, proficient MMR; SAR, survival after recurrence; WT, wild-type.

[a] Based on direct adjusted survival curves from the Cox proportional hazards model.

[b] Adjusted for age, sex, performance score, T and N stage, primary tumor site, histologic grade, biomarkers (when applicable), and time to recurrence. Stratified Cox proportional hazards models are used with 4 treatment arms as individual strata.

and III cancers from the NSABP C-07 and C-08 adjuvant studies where dMMR was associated with a lower rate of tumor recurrence,[23] a worse SAR was seen for patients with dMMR colon cancers, although the analysis was not adjusted for *BRAF*. The authors, however, postulated that the association of dMMR with shorter SAR was due to mutant $BRAF^{V600E}$ because patients with mutant $BRAF^{V600E}$ tumors had a significantly shorter SAR.[23] In another study of patients with stages I to IV colorectal cancers,[31] transcriptomic data were used to categorize tumors into 4 consensus molecular subtypes (CMSs). The CMS I subtype was enriched for tumors with high-frequency MSI and $BRAF^{V600E}$ mutations, and patients with these tumors had a poorer SAR compared with the other 3 subtypes (CMS I-III) by univariate analysis.[31] However, the study data used to generate CMSs were not adjusted for *BRAF* (or *KRAS*) status or for TTR, which was strongly associated with SAR as shown in our data set.

We observed a statistically significant interaction between biomarkers (MMR and *KRAS*) and the primary tumor site for SAR. The significant association of dMMR with better SAR was limited to cancers of the proximal vs distal colon. Although not prognostic overall, analysis of *KRAS* mutations by primary tumor site revealed a significantly shorter SAR for patients with distal but not proximal cancers. This finding for SAR is consistent with TTR data from the N0147 cohort, in which the association of *KRAS* mutations with TTR and overall survival was stronger in patients with distal cancers.[6] Conversely and relevant to anti-epidermal growth factor receptor therapy, patients whose tumors had WT *KRAS* alleles had significantly better SAR for distal vs proximal cancers. However, patients with stage III tumors with WT *KRAS* treated with FOLFOX + cetuximab vs FOLFOX alone had similar SAR, irrespective of tumor site. In patients with metastatic colorectal cancer, a recent report suggests that distal cancers respond more favorably to cetuximab than do proximal tumors (CALGB [Cancer and Leukemia Group B] 80405).[32] Patients whose tumors harbored $BRAF^{V600E}$ mutations had significantly poorer SAR independent of primary site, but the association was stronger for distal tumors. In this report, an association between the primary tumor site and SAR was also seen in patients with stage III colon cancer treated with nonoxaliplatin-containing chemotherapy in the PETACC-3 study.[33] Factors not studied in our report that may contribute to observed differences in prognosis by tumor site include epigenetic[16] and/or other genomic[33] alterations that may be embryologically influenced because the origin of the proximal colon is from the midgut and that of the distal colon is from the hindgut. In addition, gut microbial composition or metabolites may be relevant factors. Analysis of the

associations between biomarkers and SAR by study treatment arm revealed that the better SAR for patients with dMMR tumors seen among FOLFOX-treated patients did not extend to those who also received cetuximab for reasons that are unclear. Owing to the modest number of patients with complete biomarker data in the C-08 trial, results for SAR from the FOLFOX + bevacizumab study arm are not reported.

### Strengths and Limitations

Strengths of our study include the 2 clinical trial cohorts receiving standard adjuvant FOLFOX-based chemotherapy with mature recurrence and survival data. All molecular analyses were performed on prospectively collected biospecimens. Our study findings are relevant to clinical practice in that National Comprehensive Cancer Network guidelines[34] and a forthcoming consensus guideline[30] recommend testing of all newly diagnosed colorectal cancers for expanded *RAS* and *BRAF*$^{V600E}$ mutations in combination with MMR or MSI for prognostic stratification and identification of patients with Lynch syndrome. Study limitations include the fact that biomarkers were analyzed in only a subset of the C-08 cohort and that *KRAS* testing was limited to exon 2. However, a recent study[35] found that clinicopathologic features, survival outcomes, and gene expression profiles were similar between patients whose colorectal cancer harbored *KRAS* codon 12 and 13 mutations and those with mutations at *KRAS* codons 61 and 146 or the neuroblastoma RAS viral oncogene homolog (*NRAS* [HGNC 7989]) mutations. Analysis of biomarkers by tumor site for SAR resulted in some small patient subsets for which cautious interpretation of the data are warranted. Last, no data were available on patient treatment after tumor recurrence, for which we cannot exclude an effect on SAR.

## Conclusions

The association of dMMR with more favorable SAR suggests that some of these patients may be candidates for an aggressive surgical approach at recurrence. Furthermore, therapy with an immune checkpoint inhibitor is a new therapeutic option in patients with metastatic dMMR or MSI colorectal cancers, in which impressive tumor response rates and extended progression-free survival were observed.[36] In patients with dMMR and pMMR tumors, *BRAF*$^{V600E}$ mutations were associated with significantly poorer SAR, indicating the need for novel therapies in this subset.[36,37] The significant interactions of MMR and *KRAS* mutation status with SAR by primary tumor site indicate that these biomarkers should be interpreted in this context. Taken together, these data have important implications for patients with stage III colon cancer at the time of tumor recurrence, when they can be used to inform clinical decision making.

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